

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY


(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

REC'D 07 NOV 2005

WIPO

PCT

Applicant's or agent's file reference P103239WO/SW	FOR FURTHER ACTION		See Form PCT/PEA/416
International application No. PCT/GB2004/003326	International filing date (day/month/year) 30.07.2004	Priority date (day/month/year) 31.07.2003	
International Patent Classification (IPC) or national classification and IPC C07K14/82, C12N15/11, C12N15/12, C07K16/32, A61P35/00			
Applicant MILNER, PROFESSOR JO et al.			
<p>1. This report is the International preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 7 sheets, including this cover sheet.</p> <p>3. This report is also accompanied by ANNEXES, comprising:</p> <p style="margin-left: 20px;">a. <input checked="" type="checkbox"/> sent to the applicant and to the International Bureau) a total of 4 sheets, as follows:</p> <p style="margin-left: 40px;"><input checked="" type="checkbox"/> sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).</p> <p style="margin-left: 40px;"><input type="checkbox"/> sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the International application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.</p> <p style="margin-left: 20px;">b. <input type="checkbox"/> (sent to the International Bureau only) a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).</p>			
<p>4. This report contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input type="checkbox"/> Box No. II Priority</p> <p><input checked="" type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p><input type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input type="checkbox"/> Box No. VIII Certain observations on the international application</p>			
Date of submission of the demand 31.05.2005		Date of completion of this report 07.11.2005	
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016		Authorized Officer Andres, S Telephone No. +31 70 340-2671	



**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2004/003326

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
- ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
- ☐ international search (under Rules 12.3 and 23.1(b))
 - ☐ publication of the international application (under Rule 12.4)
 - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-17 as originally filed

Sequence listings part of the description, Pages

1-7 as originally filed

Claims, Numbers

1-22 received on 06.06.2005 with letter of 31.05.2005

Drawings, Sheets

1/7-7/7 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):
4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
- ☐ the description, pages
 - ☐ the claims, Nos.
 - ☐ the drawings, sheets/figs
 - ☐ the sequence listing (*specify*):
 - ☐ any table(s) related to sequence listing (*specify*):

* If item 4 applies, some or all of these sheets may be marked "superseded."

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2004/003326

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-10,16,18,20

because:

☒ the said international application, or the said claims Nos. 1-9 (as far as industrial applicability is concerned) relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 10,16,18,20 are so unclear that no meaningful opinion could be formed (*specify*):

see separate sheet

☒ the claims, or said claims Nos. 10,16,18,20 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form

☐ has not been furnished

☐ does not comply with the standard

the computer readable form

☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2004/003326

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	6
	No: Claims	1-5,7-22
Inventive step (IS)	Yes: Claims	6
	No: Claims	1-5,7-22
Industrial applicability (IA)	Yes: Claims	11-15,17,19,21 and 22
	No: Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

**INTERNATIONAL PRELIMINARY REPORT
ON PATENTABILITY**

International application No.
PCT/GB2004/003326

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☐ contained in the international application as filed
 - ☐ filed together with the international application in computer readable form
 - ☒ furnished subsequently to this Authority for the purposes of search and/or examination
 - ☒ received by this Authority as an amendment on
2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional observations, if necessary:

Prior art:

- D1: CANCER GENE THERAPY, vol. 10, no. 2, (February 2003), pages 125-133 [XP002293680]
D2: US-B1-6 414 134 (2 July 2002)
D3: NUCLEIC ACIDS RESEARCH SUPPLEMENT, no. 2, (January 2002), pages 251-252 [XP002968175]
D4: JOURNAL OF THE NATIONAL CANCER INSTITUTE, vol. 93, no. 6, (21 March 2001), pages 463-471 [XP009003270]
-

Section III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

- III.1. Claims 1-10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).
- III.2. Claims 10,16,18 and 20 relate to compounds defined by reference to a desirable characteristic or property, namely their capacity to bind an abnormally spliced bcl-2 gene or protein. The claims cover all compounds having this characteristic or property (most probably including known compounds; See also Section V.), whereas the application provides support and/or disclosure within the meaning of Articles 5 and 6 PCT for none of such compounds. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Therefore, no meaningful opinion can be given on said claims.

Section V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1. NOVELTY (Art. 33(2) PCT) and INVENTIVE STEP (Art. 33(3) PCT)

- V.1.1. Inhibitory nucleic acid molecules targeting regions of the bcl-2 gene common to all (see D1 and D3) or only the beta splice-variants (D2 and D4) were disclosed in the prior art. Documents D1-D4, therefore anticipate the subject-matter of claims 1,2,4,5,7-15,17,19,21 and 22 which do not fulfill the requirements of Art. 33 (2) PCT.
- V.1.2. Concerning agents targeting the **protein** product of the abnormally spliced variants of bcl-2 (claims 3,16,18 and 20), besides the fact that no such agent has been characterised in the application, it has to be noted that antibodies to the wild-type bcl-2 gene could fall within the scope of the claims which are therefore not considered as novel in the sense of Art. 33(2) PCT.
- V.1.3. The method as characterised in claim 6 is novel and inventive (Art. 33(3) PCT), as none of the available prior art documents discloses or suggests the inhibition of that particular bcl-2 α variant.

V.2. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT)

For the assessment of the present claims 1-10 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims

1. A method of regulating apoptosis in a cell, said method comprising targeting an abnormally alternatively spliced Bcl-2 mRNA , an abnormally alternatively structured Bcl-2 mRNA, or a product of either.
5
2. A method according to claim 1 comprising targeting the junctions of the Bcl-2 mRNA molecule that is abnormally spliced or abnormally structured.
3. A method according to claim 1 comprising targeting a protein product following
10 translation of the abnormally spliced or abnormally structured Bcl-2 mRNA.
4. A method according to any of claims 1 to 3 comprising the selective silencing of abnormal splice variants of the Bcl-2 gene.
- 15 5. A method according to claim 4 comprising the targeting of any of the abnormal splice variants selected from the group consisting of: Bcl-2 α -591, Bcl-2 α -588, Bcl-2 α -480, Bcl-2 α -633, Bcl-2 β -489, Bcl-2 β -474, Bcl-2 β -420 and/or Bcl-2 β -315.
6. A method according to claim 5 comprising targeting of the mRNA sequence
20 flanking the splice junction between nucleotides 111 and 241 of Bcl-2 α -591.
7. A method according to any of the preceding claims comprising targeting an abnormally spliced Bcl-2 mRNA or a product thereof, by introducing into a cell containing a Bcl-2 gene which is abnormally spliced and which is to be targeted, an RNA
25 construct having a nucleotide sequence which is homologous to mRNA within said cell wherein said mRNA includes genetic information of the Bcl-2 gene element that is abnormally spliced.
8. A method according to claim 7 wherein the RNA construct is a small interfering
30 dsRNA (siRNA).
9. A method according to claim 8 wherein the siRNA is up to 28 nucleotides long.

10. A method according to any of claims 1 to 6, comprising targeting an abnormally spliced Bcl-2 mRNA or a product thereof, by introducing into a cell containing a Bcl-2 gene which is abnormally spliced and which is to be targeted, an agent selected from the group consisting of: small molecule or protein; polypeptide; peptide; aptamer; chemical; antibody; nucleic acid; polypeptide or nucleotide probe; anti-sense RNA; shRNA; miRNA; and Bcl-2 derived products including abnormal Bcl-2 splice variants which inhibit Bcl-2 either directly or indirectly; which agent interacts with or binds with the abnormally spliced Bcl-2 mRNA or protein expressed by the abnormally spliced Bcl-2 mRNA.
11. A nucleotide construct with a nucleotide sequence which is at least 50% homologous to mRNA transcribed from an abnormally spliced Bcl-2 gene.
12. A nucleotide construct according to claim 11 wherein said construct comprises dsRNA.
13. A nucleotide construct according to claim 12 wherein the construct is 20 to 28 nucleotides long.
14. A nucleotide construct according to claim 13 wherein the RNA construct is 21 to 22 nucleotides long.
15. A nucleotide construct selected from the group consisting of: siRNA; anti-sense RNA; shRNA; and miRNA; as means for silencing the expression of an abnormally spliced Bcl-2 gene for use as a medicament.
16. An agent selected from the group consisting of: small molecule or protein; polypeptide; peptide; aptamer; chemical; antibody; nucleic acid; polypeptide; and nucleotide probe; which agent interacts with or binds with a protein expressed by an abnormally spliced Bcl-2 mRNA, for use as a medicament.
17. Use of a nucleotide construct selected from the group consisting of: siRNA; anti-

sense RNA; shRNA; and miRNA; and capable of silencing the expression of an abnormally spliced Bcl-2 gene for the manufacture of a medicament for the treatment of cancerous cell growth.

- 5 18. Use of an agent selected from the group consisting of: small molecule or protein; polypeptide; peptide; aptamer; chemical; antibody; nucleic acid; polypeptide; and nucleotide probe; which agent interacts with or binds with a protein expressed by an abnormally spliced Bcl-2 mRNA, for the manufacture of a medicament for the treatment of cancerous cell growth.

10

19. A pharmaceutical composition comprising a nucleotide construct capable of silencing the expression of an abnormally spliced Bcl-2 gene and selected from the group consisting of: siRNA; anti-sense RNA; shRNA; and miRNA; and a pharmaceutically acceptable diluent or carrier.

15

20. A pharmaceutical composition comprising an agent selected from the group consisting of: small molecule or protein; polypeptide; peptide; aptamer; chemical; antibody; nucleic acid; polypeptide; and nucleotide probe; which agent interacts with or binds with a protein expressed by an abnormally spliced Bcl-2 mRNA, and a pharmaceutically acceptable diluent or carrier.

20

21. Use of a DNA or RNA expression vector as a delivery means for a molecule which is used in the targeting of an abnormally spliced Bcl-2 mRNA or a product thereof.

- 25 22. A DNA or RNA expression vector comprising an expression cassette including the nucleotide sequence selected from the group consisting of;

a) the nucleic acid sequence of an abnormally spliced gene element as shown in Fig 1;

b) a nucleic acid molecule which has at least 50% homology to and hybridizes to a

30 nucleic acid sequence of (a) ;

4

c) a nucleic acid molecule which has a nucleic acid sequence which is degenerate because of the genetic code to the sequences in a) and b) and any sequence which is at least 50% complimentary to any of the above sequences;
wherein the expression cassette is transcriptionally linked to a promoter sequence.